Angiomyolipoma: A link between stemness and tumorigenesis in the kidney.

The link between stemness and tumourigenesis in the kidney

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The origin of angiomyolipoma and of other tuberous sclerosis associated neoplasms is still unknown. Two studies show that they derive from a a multipotent cancer stem cell originated from a multipotent renal epithelium, providing a link between stemness and tumourigenesis also in the kidney.

Tuberous sclerosis complex (TSC) is a multisystem disorder caused by mutations in one of two genes, *TSC1* and *TSC2*, that affects about 1 in 6000 individuals and is characterized by mostly benign tumours and other type of lesions that can develop in multiple organs (1). In the kidneys, cysts, renal cell carcinomas, and particularly angiomyolipoma (AML) can occur (1). Renal AML are benign neoplasms that contain variable admixtures of tumour cells histologically and molecularly similar to vascular (angio-), smooth muscle (myo-) and fat (lipo-) lineages (1,2). AMLs are observed in the majority of patients with TSC, but can also arise sporadically, affecting ~0.6% of females and 0.3% of males in the general population (1,2).

Genetic analyses have shown that AML are clonal (3), indicating they originate from a single tumour-initiating cell with the capacity to differentiate into all these distinct lineages, but the mechanisms behind this plasticity are unclear. As embryonic neural crest stem cells or neural crest-derived progenitor cells from the adult skin can differentiate to form melanocytes, adipocytes, and smooth muscle cells, the cell type of origin of renal AML has been proposed to be an unidentified kidney-resident cell, neural crest-derived lineage, myoblasts, pericytes or lymphatic endothelium (1,4)

Two studies recently published in *Nature Communications* show that the source of this plasticity resides in the derivation of these tumors from a cancer stem cell (CSC) originating from the renal epithelium (5,6) (Figure 1). Indeed, Goncalves *et al.* showed that *TSC2* knockdown transforms senescence-resistant cultured mouse and human renal epithelial cells into neoplastic stem cells (SC) that serially propagate renal AML-like tumours in mice (5). In addition, deletion of *Tsc1* in mouse renal epithelia causes differentiation *in vivo* into cells expressing characteristic AML markers (5). Consistently, Cho *et al.* showed that hyperactivation of a Rheb-Notch-Rheb regulatory loop is a key event to block cell differentiation of the multi-lineage, SC characteristics of AML cells, maintaining their multipotency (6) (Figure 1).

The observation that the multiple cell lineages AML derive from a CSC directly generated from a proximal tubular epithelial cell is intriguing and somehow surprising. Indeed, this observation suggests that tubular epithelium can potentially display multidifferentiation capacity, going beyond the epithelial phenotype that has been considered its only possible option. However, under certain circumstances a subset of renal epithelial cells exhibits cellular plasticity and displays progenitor- or SC-like properties (7,8) (Figure 1). Indeed, both Goncalves et al. and Cho et al. suggest the potential origin for TSC-associated renal cell carcinoma (RCC) from renal epithelial cells localized in the Bowman's capsule urinary pole. Isolation of this population by cell sorting using two markers that specifically characterize it, CD133 and CD24, from adult human kidney tissues, revealed that these cells can grow as sphere and resist senescence and could be differentiated in culture into

several types of renal epithelial cells, adipocytes, osteoblasts, endothelial cells and neuronal cells, suggestive of a multi-potent progenitor cell phenotype (7,8). *In vivo* transplantation of clonal populations in different mice models of kidney injury showed that these cells can generate endothelium as well as different types of tubular epithelimun in acute tubular injury, where mostly vascular and tubular cells are damaged, or podocytes and tubular epithelium in adriamycin nephropathy, where injury involves podocytes and tubular cells of different segments (7,8). Of note, these properties were not shared by any other epithelial cell of the kidney used as controls for *in vitro* and *in vivo* experiments (7,8).

Although multipotent progenitors exist in the adult kidney, when lineage tracing techniques are applied to renal epithelium, homeostasis and regeneration after acute kidney injury only involves unipotent progenitors specific for each tubule segments (9) (Figure 1). This apparent discrepancy is not surprising. The behavior of normal adult SC assessed by lineage tracing differs substantially from that inferred through cell culture or transplantation experiments (10). Hair-follicle SC give rise to all epidermal lineages upon transplantation, but upon lineage tracing generate only hair-follicle lineages (10). Similarly, although mammary basal cells are multipotent in transplantation assays, these cells are unipotent when interrogated by lineage tracing (10). Overall, these observations imply that cell culture experiments and transplantation-based approaches may reveal the potential of SC, but the fate of these cells in their own environment *in vivo* maybe different and largely restricted by factors produced by the niche (10). These observations have prompted a revision of the classical SC model, that is based on premises and concepts inherited from the canonical hardwired SC/CSC hierarchy (10). In this type of cell hierarchy, SC/CSCs are rare, relatively quiescent and largely defined by intrinsic properties, such as the capacity to undergo asymmetric division, to generate one SC and one more differentiated cells (10). By contrast, organ adult SC can be abundant in their niches (e.g. up to 10% of crypt cells are intestinal SC) and a SC division can result in zero, one, or two new SC, depending on the available niche space, a process known as neutral competition (10). In this perspective, not only genetic alterations, but also signals coming from the

niche or the microenvironment can modulate plasticity and differentiation capacity of resident adult SC, even promoting their transfromation from SC to CSC (10).

The studies of Goncalves *et al.* and Cho *et al.* suggest that signals of the kidney environment in the context of *TSC1* or *-2* gene deletion modulate differentiation of a resident epithelial SC to prime its multipotent undifferentiated state and transform it in a CSC that generates AML or RCC (5,6) (Figure 1). Intriguingly, Cho *et al.* also report that cell-specific loss of *TSC1* within neuroepithelial-crest derived precursors, labelled as nestin-expressing cells in adult mice, leads to the formation of kidney cysts, renal intraepithelial neoplasia and papillary renal carcinoma, suggesting that other TSC-associated tumors may share a similar pathogenesis.

Although more thorough investigations are required to fully support this notion, these studies provide the first direct demonstration of the importance of CSC for the pathogenesis of at least some subtypes of renal neoplasms and underline the strict link between stemness and tumourigenesis even in the kidney. Several pharmaceutical companies have launched programs aimed at eliminating CSC or at modulating their function and plasticity by modulating the tumor niche (10). These new findings open to the potential to explore these possibilities also for the kidney.

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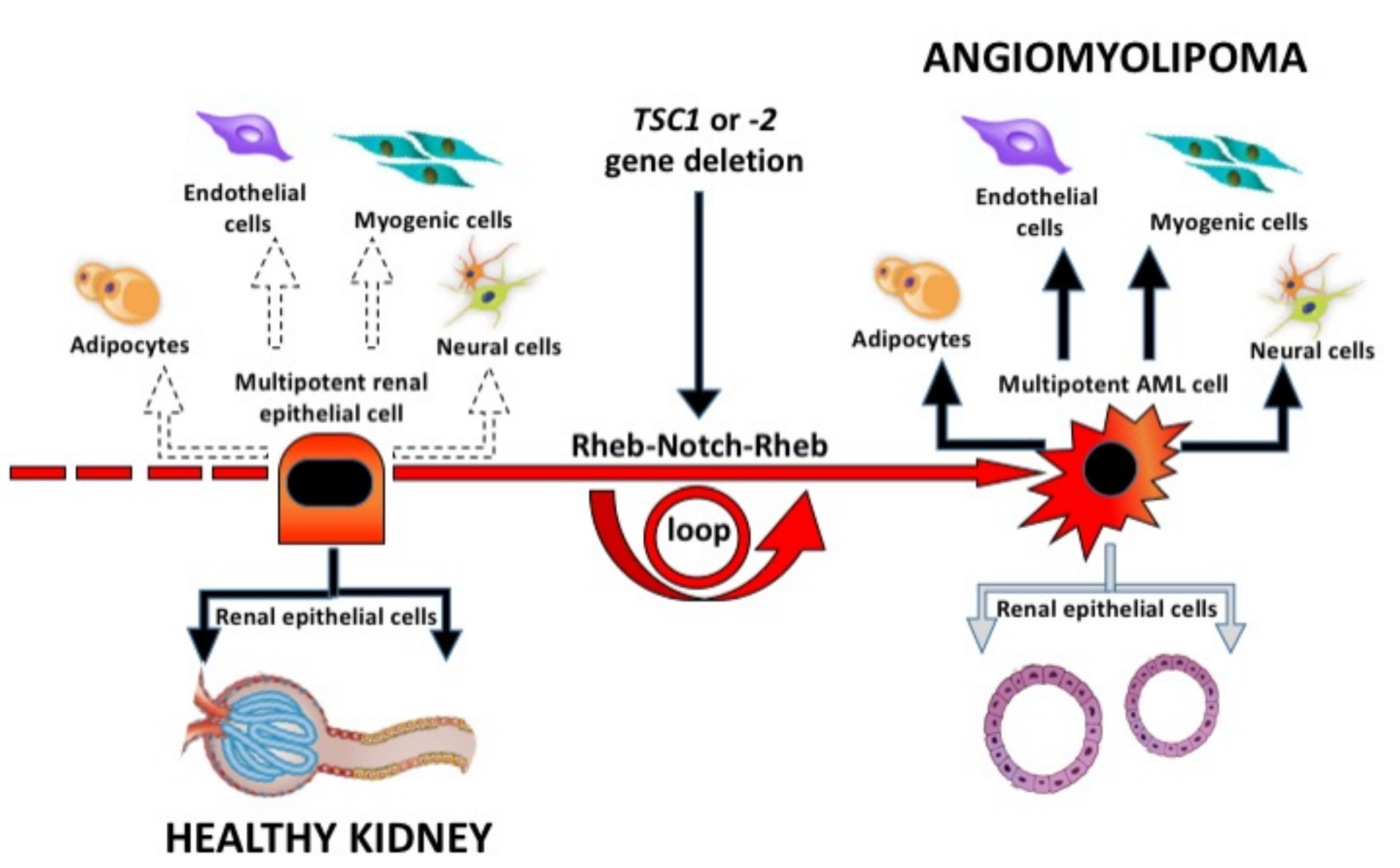
LEGENDFOR FIGURES

Figure 1. Scheme summarizing the link between stemness and tumorigenesis in the kidney

Renal epithelial cells showing multilineage differentiation potential have been described in the adult kidney. After kidney injury these cells can only differentiate into other epithelial cells. However, angiomyolipoma, that is clonally generated by multipotent renal epithelial cells, shows multilineage differentiation potential not only in cells of the epithelial renal lineage, but also into adipocyte, vascular cells, myocytes and neural cells that can all be observed inside this tumor. A Rheb-Notch-Rheb loop, activated upon TSC1 or -2 gene deletion, controls the undifferentiated “stemness” status of angiomyolipoma cells.

AML, angiomyolipoma; TSC, Tuberous Sclerosis Complex.


ANGIOMYOLIPOMA
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